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New metallic compounds with ligands derived from Hpyrimol. Study of their interaction with DNA.

Nous compostos metàl·lics amb lligands derivats del Hpirimol. Estudi de la seva interacció amb l'ADN.

Aida López Espinar

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La investigació de les malalties ha avançat tant que és cada cop més difícil trobar a algú que estigui completament sa.

Aldous Huxley

A la Dra. Amparo Caubet Marin per tot l'interès, ajuda, consells i confiança que ha dipositat en mi durant aquests mesos de recerca.

Als meus companys de laboratori especialment al Marcos Palmeira per haver-me ensenyat molts coneixements nous.

Als meus pares per recolzar-me sempre en totes les decisions que decideixo prendre.

Als meus amics i amigues pels ànims, riures i bons moments que hem passat tot i els nervis patits durant aquest semestre.

REPORT

CONTENTS

1. SUMMARY

The new ligands (*E*)-4-(((pyridine-2-ylmethyl)imino)methyl)aniline (**L1**) and (*E*)-2-(((5-bromopyridin-2-yl)methylene)amino)phenol (**L2OH**) have been prepared by the condensation of 4-aminobenzaldehyde and 2-pycolylamine or 5-bromopycolynaldehyde and 2-aminophenol respectively. The reaction of L1 with Na_2PdCl_4 produce a mixture of the compounds $[PdCl_2(L1)]$ and $[PdCl_2(2\text{-pycolylamine})]$, this last one due to the ligand hydrolysis. The presence of a phenolic ring with a hydroxyl group susceptible to be deprotonated in ligand L2OH causes that the reaction of this ligand with Na_2PdCl_4 or $[PtCl_2(DMSO)_2]$ produce a mixture of the compounds $[MCl(L2O)]$ and $[MCl_2(L2OH)]$, {M = Pd(II), Pt(II)}. The ligand L2OH acts as a (N,N',O) tridentate donor group in the former one compound or as a (N,N') bidentate ligand in the second one. Modifying the pH it is possible to obtain pure compounds, but the $[MCl_2(L2OH)]$ complexes synthesized are very unstable in solution.

The ligands and the metallic compounds were characterized by elemental analysis, infrared spectroscopy (IR) and mono- and two-dimensional NMR spectroscopies. It was also possible to determinate the crystalline structure of L2OH by X-ray diffraction.

The interaction with DNA was studied for $[MCl(L2O)]$ {M = Pd(II), Pt(II)} and $[PtCl_2(L2OH)]$ complexes by UV-Vis and fluorescence spectroscopies, circular dichroism and electrophoresis.

The complexes that, in general, present more interaction with DNA are [PdCl(L2O) and $[PtCl_2(L2OH)]$].

Keywords: Pd(II) and Pt(II) complexes, DNA interaction, Schiff bases.

2. RESUM

Els nous lligands *(E)*-4-(((piridina-2-ilmetil)imino)metil)anilina (**L1**) i *(E)*-2-(((5-bromopiridina-2-il)metilen)amino)fenol (**L2OH**) s'han preparat mitjançant la condensació de 4-aminobenzaldehid i 2-picolilamina o 5-bromopicolilaldehid i 2-aminofenol respectivament. La reacció de L1 amb Na_2PdCl_4 produeix una barreja dels compostos $[PdCl_2(L1)]$ i $[PdCl_2$(2-picolilamina)], aquest últim a causa de la hidròlisi del lligand. La presència d'un anell fenòlic amb un grup hidroxil susceptible a ser desprotonat en el lligand L2OH fa que la reacció d'aquest lligand amb Na_2PdCl_4 o $[PtCl_2(DMSO)_2]$ produeixi una barreja dels compostos $[MCl(L2O)]$ i $[MCl_2(L2OH)]$, {M = Pd(II), Pt(II)}, on L2OH actua com a grup donador (N, N ', O) tridentat en el primer cas o com a lligand (N, N') bidentat en el segon. La modificació del pH fa possible obtenir els compostos purs, però els complexos $[MCl_2(L2OH)]$ són molt inestables en solució.

Els lligands i els compostos metàl·lics es van caracteritzar per anàlisi elementals, espectroscòpia infraroja (IR) i RMN mono i bidimensional. També es va poder determinar l'estructura cristal·lina de L2OH mitjançant difracció de raigs X.

Es va estudiar la interacció amb l'ADN per als complexos $[MCl(L2O)]$ {M = Pd(II), Pt(II)} i $[PtCl_2(L2OH)]$ per espectroscòpies UV-Vis i fluorescència, dicroisme circular i electroforesi.

Els complexos que, en general, presenten més interacció amb l'ADN són $[PdCl(L2O)]$ i $[PtCl_2(L2OH)]$].

Paraules clau: Complexos de Pd(II) i Pt(II), interacció and l'ADN, bases de Schiff.

3. INTRODUCTION

Cancer is a set of diseases in which the body produces an excess of malignant cells (known as cancerous), which growth beyond the normal limits that invade healthy tissues. These cells affect organs and tissues due to rapid expansion through lymphatic system or circulatory; this expansion is called metastasis[1].

Many cancers form solid tumors, which are an accumulation of cancerous cells but blood cancers, such as leukemia, generally do not form solid tumors.

Nowadays there are some treatments to overcome it: radiotherapy which uses X-rays or γ-rays, surgery to remove the tumor and chemotherapy which consist in the administration of several drugs to eliminate the cancerous cells. There are two types of drugs in chemotherapy: ones that inhibit DNA synthesis (like cisplatin) and others that act on cytoplasmic compounds that are necessary in cell division[1].

One of the most used anticancer drugs is *cis*-diamminedichloridoplatinum(II), cisplatin (Fig.1a), which antitumor properties were discovered by Barnett Rosenberg in 1960. It is routinely used for the treatment of testicular and ovarian cancer, and is increasingly used against others tumors, such as cervical, bladder and head/neck tumors[2]. However, its use is limited by the severe side effects that it presents like neurotoxicity and nephrotoxicity, its low solubility and the appearance of resistance, both acquired and intrinsic[3].

The primary cisplatin target is DNA. In the blood stream where the chloride concentration is relatively high (100 mM) the chloride ligands stay attached to the drug. When it reaches the tumor is thought that enters to the cell by passive diffusion or by active protein-mediated transport systems. Ones inside the cell, the lower chloride concentration (4-20 mM) in the cytoplasm produce a drug aquation with the loss of one or both of chloride ligands and can go on to bind to DNA[4]. Cisplatin will bind at the N7 position of two neighbouring guanine bases and to a lesser extent adenine, forming a wild range of adducts, particularly 1,2-GpG intrastrand adducts producing a kink of the DNA structure. This DNA distortion prevents replication and transcription, which ultimately leads to cellular apoptosis (cell death)[2].

Of the thousands of complexes tested over the years in the quest for new platinum drugs that would perform better than cisplatin, very few have entered clinical trials: carboplatin and oxaliplatin (Fig. 1b and 1c respectively) were approved for clinical use worldwide, whereas nedaplatin, lobaplatin and heptaplatin (Fig. 1d, 1e, and 1f) were only approved for use in Japan, China and South Korea, respectively. Carboplatin is used for the same cancers that cisplatin but it has less toxicity so it can be used in higher doses and oxaliplatin is used for colorectal cancer[3].

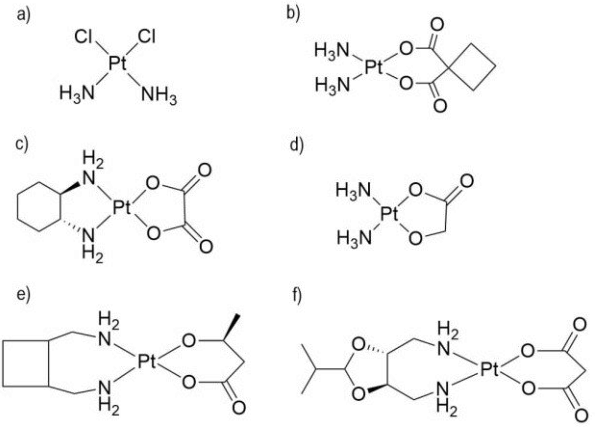


Figure 1. a) Cisplatin, b) carboplatin, c) oxaliplatin, d) nedaplatin, e) lobaplatin, f) heptaplatin.

Although these compounds undoubtedly present an improvement over cisplatin in terms of side-effects (carboplatin) and selectivity (oxaliplatin) they suffer from similar drawbacks, albeit in less extent. Attempts to overcome these shortcomings have results in different compounds: i) non-classical platinum complexes or another metallic complexes with different DNA-binding modes, ii) Pt-complexes that accumulate at the tumor site by a drug targeting and delivery strategy, iii) Pt-complexes that are activated only in tumor tissues or cells (prodrugs)[5]. Consequently, the development of new drugs is still required.

Because of the similarities between Pt(II) and Pd(II), some palladium complexes have been also studied. Some investigations show that Pd(II) compounds have better cytotoxicity, less nephrotoxicity and higher specificity than cisplatin and its analogues. In terms of usefulness, the slow dissociation pattern of platinum complexes compared to palladium (10^5 times faster) makes

them more applicable[6]. A recent trend observed in palladium-based anticancer drugs is to focus on the development of palladium (II) complexes with slower rates of hydrolysis choosing polidentate nitrogen ligands and suitable leaving groups[7].

The imines or Schiff bases form stable complexes with transition metals. They have been taken importance as a ligands due to their preparative accessibilities, varied denticities (forming a five-membered chelate ring), structural varieties, solubility in most of common solvents and thermal stability. They also have biomimetic properties because they can imitate structural characteristic of active sites[7,8]. In that context, a square-planar copper(II) complex with remarkable DNA-cleaving properties was obtained from a very simple Schiff-base ligand, namely 4-methyl-2-N-(2-pyridylmethylene)aminophenol (Hpyrimol)[9]. In addition, the use of Hpyrimol allowed the synthesis of efficient nuclease-active zinc(II) complexes, whose DNA-cleaving activities were based on the ligand, through the generation of phenoxyl radicals[10].Also the platinum compounds, [PtCl(Hpyrimol)] and [$PtCl_2$(Hpyrimol)]) have been described together with their cytotoxic properties[11,12].

In order to develop new antitumor drugs which specifically target DNA, it is necessary to understand the different binding modes that a complex is capable to undertake. Basically, metal complexes interact with the DNA double helix in either a non-covalent or a covalent way. The non-covalent way includes three binding modes: intercalation between two adjacent base pair, major or minor grove binding and electrostatic interaction[13].

To see the interactions between DNA and complexes are used different techniques: UV-Vis and fluorescence spectrometry, circular dichroism and electrophoresis. These techniques allow having an idea to see the changes that the complexes produce to DNA.

4. OBJECTIVES

In the present study, the aim is the preparation and characterisation of some metal complexes with Schiff-bases, inspired in Hpyrimol, as ligands, and study their interaction with DNA.

The specific objectives are:

- The synthesis and characterisation of new Schiff-base ligands.
- The synthesis and characterisation of palladium (II) and platinum (II) complexes.
- Study the interaction of the ligands and the complexes with DNA by UV-Vis and fluorescence spectroscopes, circular dichroism and electrophoresis.

The ligands have been designed to be able to functionalize nanoparticles, so that subsequently, they can transport the drugs. Various types of nanoparticles (NPs), including inorganic ones (such as gold NPs), can be used as drugs carriers.

5. RESULTS AND DISCUSSION

5.1. LIGANDS SYNTHESIS AND CHARACTERISATION

The general method used to prepare a Schiff base or imine is a condensation between an aldehyde or a ketone and a primary amine (Fig. 2)[14].

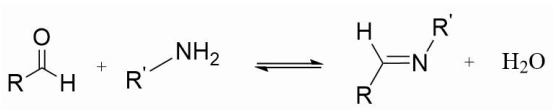


Figure 2. General reaction of condensation.

This reaction is an equilibrium so the product can be hydrolysed easily. The product could be in the *E* or *Z* form. The stability of the formed isomer depends on the sterical hindrance between the R groups. Generally, the most stable product is the *E* form for N-substituted imines so it will be considered that this is the isomer obtained.

The condensation of 4-aminobenzaldehyde and 2-pycolylamine or 5-bromopycolynaldehyde and 2-aminophenol using ethanol as a solvent allows to obtain the ligands (*E*)-4-(((pyridine-2-ylmethyl)imino)methyl)aniline (**L1**) or (*E*)-2-(((5-bromopyridin-2-yl)methylene)amino)phenol (**L2OH**), respectively, in good yields (Fig. 3).

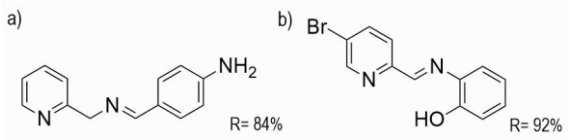


Figure 3. a) (E)-4-(((pyridine-2-ylmethyl)imino)methyl)aniline (**L1)** and b) (*E*)-2-(((5-bromopyridin-2-yl)methylene)amino)phenol (**L2OH**).

The Schiff bases were characterized by elemental analysis, IR and mono- and two-dimensional NMR spectroscopies (see Experimental section). The IR spectra of both ligands show the characteristic band at around 1600 cm^{-1} due to the v(C=N) group. Furthermore, it can be seen the bands of the substituent groups (v(N-H) at 3300 cm^{-1} for L1 and v(O-H) at 3349 cm^{-1} for L2OH).

One- and two-dimensional NMR experiments suggest that only one isomer (*E*-form) is present in solution. The common feature in the ^{1}H-NMR spectra of Schiff bases is the imine proton signal which appears as a singlet in the range 8.0-9.0 ppm (δ= 8.2 ppm for L1 and δ= 8.7 ppm for L2OH) and is indicative that the imine bond is formed.

For the ligand L1 it can also be observed the signal corresponding to the amine group of the phenyl ring as a singlet at 5.6 ppm. This fact indicates that no condensation has occurred between molecules of 4-aminobenzaldehyde.

Crystals of L2OH suitable for X-ray analyses could be obtained by diffusion of diethyl ether into a MeOH solution of the ligand. The molecular structure with the corresponding atom labelling is shown in Fig. 4 and crystallographic and refinement parameters are summarised in Appendix 3.

The structure consists of discrete molecules which interact with two adjacent molecules by hydrogen bond. The distance between the oxygen (O_1) and the hydrogen of a neighbouring molecule is 2.25 Å.

The distance of C7-N1 bond [1.273(2) Å] is similar to other imines found in the literature[15]. This distance and the C8-C7-N1 bond angle [121.38(17) º] illustrate the double bond character and the sp^2 hybridization of the imine carbon atom. The single bond N1-C6 has a longer distance [1.411(2) Å] than the imine C7-N1 bond.

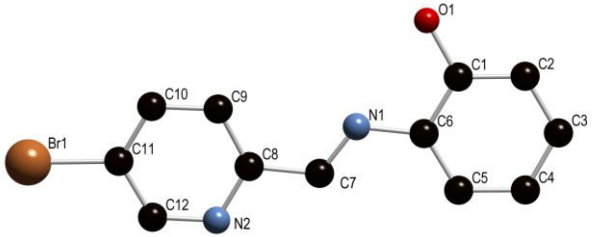


Figure 4. Representation of the molecular structure of L2OH with the corresponding atom labelling. The hydrogen atoms are omitted for clarity. Selected bond lengths are C7-N1 1.273(2) Å; C8-N2 1.345(2) Å and C7-N1 1.411(2) Å and angles C7-C8-N1 121.38(17) º and the dihedral angle between C6-N1-C7-C8 179.8 º.

The value of the torsion angle C8-C7-N1-C6 (179.8º) indicate that the imine adopts the anti-(*E*) conformation[16].

The phenyl and pyridine rings are planar, and they are practically coplanar.

5.2. METAL COMPLEXES SYNTHESIS AND CHARACTERISATION

The reaction in methanol of stoichiometric amounts of $[PtCl_2(DMSO)_2]$ or Na_2PdCl_4, previously dissolved and filtered, and the ligand L2OH produces a mixture of two products: $[MCl_2(L2OH)]$ {where L2OH acts as a (N,N') bidentate ligand} and [MCl(L2O)] {where L2OH acts as a tridentate (N,N',O) donor group} {M= Pd(II), Pt(II)} (Fig. 5).

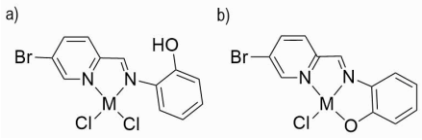


Figure 5. Metallic complexes obtained {M = Pd(II); Pt(II)}. a) $[MCl_2(L2OH)]$, b) [MCl(L2O)].

In order to obtain the two pure compounds, the pH of the reaction was modified. Adding stoichiometric amounts of hydrochloric acid the complex $[MCl_2(L2OH)]$ should be obtained, while the addition of a base like triethylamine should lead to the complex [MCl(L2O)].

All pure complexes have been characterized by the usual techniques of elemental analysis, IR and NMR spectroscopies.

When the reaction is carried out under basic conditions only the expected product is obtained {[MCl(L2O)]} {M = Pd(II) or Pt(II)}. The ^1H NMR spectra of both complexes are consistent with the proposed structures. The proton signals more shifter compared with those of the free ligand are the imine and the alpha proton of pyridine (H^6) in agreement to the ligand coordination by the nitrogen of pyridine and imine. The signal of this last proton appears as a doublet due to the coupling with the proton H^3 in *trans*. In these spectra it is also observed that the signal corresponding to the proton of the OH group disappears indicating that this group is coordinated.

In the ^1H NMR spectrum of [PtCl(L2O)] is possible to observe the ^1H-^{195}Pt coupling between the iminic proton and platinum. The value of the measured coupling constant is $^3J_{H7\text{-}195Pt}$ = 100 Hz that is in good agreement with those observed for other Pt(II) complexes[17].

In IR spectra the ν(C=N) band is observed at 1576 cm^{-1} for [PdCl(L2O)] and at 1591 cm^{-1} for [PtCl(L2O)].

When the reaction was carried out in acid conditions, for the platinum it was possible to obtain the expected product $[PtCl_2(L2OH)]$ pure. In the ^1H NMR spectrum only one set of signals is observed that can be assigned to the compound. In this spectrum, a difference of the case of

the compound [PtCl(L2O)], there is a singlet at 10.1 ppm which belongs to the hydroxyl group and is indicative that the ligand is not bonded to the metal through the oxygen. The analytical results agree with the proposed structure.

Although the pure [$PtCl_2$(L2OH)] compound was obtained, this compound in solution is hydrolysed in 36h.

The synthesis in acid conditions for the Pd(II) complex allows to obtain the pure solid product but in solution is quickly converted in [PdCl(L2O)]. In the ^{1}H NMR spectrum is observed the signal of the hydroxyl group at 10.1 ppm and other signals that can be assigned to the [$PdCl_2$(L2OH)] compound, but also there are signals that belong to [PdCl(L2O)] and to aldehyde and amine. In the IR spectra for both compounds appear the ν(O-H) about 3300 cm^{-1} and the ν(C=N) in 1600 cm^{-1}.

The relative situation of signals of the imine proton and the alpha proton of pyridine (H^6) in the ^{1}H NMR spectra allows the complexes [MCl_2(L2OH)] and [MCl(L2O)] to be easily identified. In the [MCl(L2O)] complexes the iminic proton signal appears at lower fields than the alpha pyridine proton whereas in the [MCl_2(L2OH)] complexes this order is inverted, the iminic proton signal appears at higher fields than the alpha pyridine proton.

The reaction in methanol of stoichiometric amounts of Na_2PdCl_4, previously dissolved and filtered, and the ligand L1 produces a mixture of products, [$PdCl_2$(L1)] and [$PdCl_2$(2-pycolylamie)]. This last product was obtained pure by crystallization of the filtrate. These products could be characterized by ^{1}H NMR.

The ^{1}H NMR spectrum shows the characteristic signal of the imine proton (δ= 8.4 ppm) of [$PdCl_2$(L1)] compound. Also is possible to assign some of the pycolylamine signals that belong to the [$PdCl_2$(2-pycolylamine)] complex and the aldehyde (δ= 9.5 ppm) of 4-aminobenzaldehyde. The presence of aldehyde indicates that the imine was hydrolysed and the resulting amine reacts with Na_2PdCl_4 to form [$PdCl_2$(2-pycolylamine)].

5.3. DNA INTERACTIONS

5.3.1. Fluorescence spectroscopy

The ability of Pd(II) and Pt(II) complexes to displace the DNA-intercalator ethidium bromide (EB) was evaluated using fluorescence spectroscopy. EB fluoresces when it is intercalated

between DNA base pairs (λem = 592 nm). If the complexes are able to displace the EB, it is expected that the fluorescence decreases. To compare the displacement ability of the complexes, their quenching efficiency was assessed applying the Stern–Volmer equation (Eq. 1)[13].

$$\frac{I_0}{I} = 1 + K_{sv}[\text{complex}]$$

Equation 1.

The corresponding Stern–Volmer quenching constant (K_{sv}), is determined by plotting I_0/I versus [complex] (I_0 and I being the emission intensities in the absence and the presence of the complex, respectively).

The fluorescence spectra were recorded at constant concentrations of EB and DNA (of respectively, 75 and 15 μM in cacodylate-NaCl buffer) upon addition and incubation for 24h at 37 °C of increasing amounts of the metal complex, namely in the 0–150 μM range. The emission intensities were measured from 530 to 700 nm with an excitation wavelength at 514 nm.

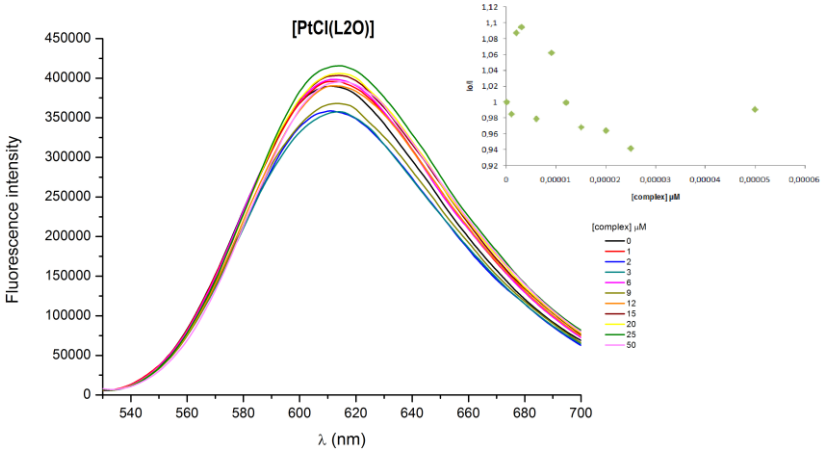


Figure 6. [PtCl(L2O)] Fluorescence spectra and I_0/I versus [complex].

The fluorescence spectrum of [PtCl(L2O)] is shown in Fig. 6. When the complex concentration increases, the fluorescence does not decrease so the constant cannot be calculated because the plot of I_0/I versus [complex] doesn't follow a lineal tendency (Insert Fig.

6). The [PdCl(L2O)] and [$PtCl_2$(L2OH)] complexes present similar behaviour (Appendix 8).That could be attributed to the no displacement of the EB by the complexes indicating they could interact through another modes of interaction, for instance groove binding. This means that EB has a bigger K_{sv} than the prepared complexes.

5.3.2. Circular dichroism (CD)

Circular dichroism (CD) is a sensitive technique to monitor the conformational changes in the DNA secondary structure upon interaction with the synthesized compounds. The CD spectrum of the DNA B form consists of a positive band at 275 nm due to base stacking and a negative band al 245 nm due to helicity[18]. This is the most common form of DNA even though exist A and C forms that show a different spectra[19].

CD spectra of DNA after the addition of compounds L2OH, [PdCl(L2O)] and [PtCl(L2O)] incubated for 24 hours al 37ºC were recorded. The effect of these compounds on the secondary structure of DNA was studied by keeping the concentration of DNA at 100 µM varying the compounds concentration (0-200 µM) in a cacodylate-NaCl buffer.

The obtained spectra are shown in Fig. 7.

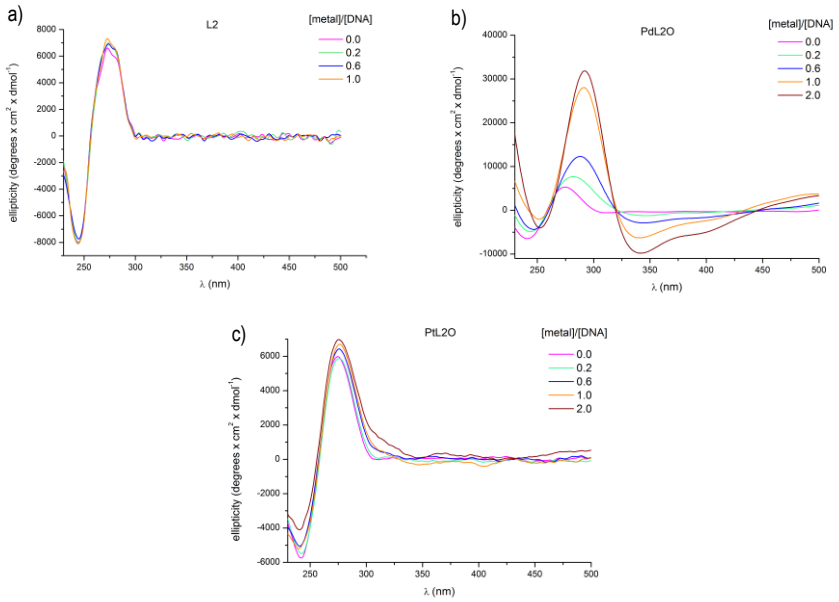


Figure 7. CD spectra of a) L2OH, b) [PdCl(L2O)] and c) [PtCl(L2O)]

The maximums and the minimums of ellipticity (θ) at different ratio [metal]/[DNA] and the wavelengths at which they appear are shown in Table 1.

Compund	[metal]/[DNA]	Θ_{max} [a]	λ_{max} [nm]	Θ_{min} [a]	λ_{min} [nm]	$\Delta\ \Theta_{max}$	$\Delta\ \Theta_{min}$
DNA	-	6.6	273	-8.0	244	-	-
L2	0.2	7.0	273	-8.0	244	0.39	0.09
	0.6	7.0	273	-7.7	245	0.31	0.25
	1	7.3	272	-8.1	244	0.72	0.11
DNA	-	6.3	273	-8.4	243	-	-
[Pd(L2O)]	0.2	8.6	281	-6.8	245	2.24	1.61
	0.6	13.7	288	-6.4	247	7.36	2.05
	1.0	31.7	292	-6.0	253	25.38	2.46
	2.0	36.4	292	-4.0	253	30.12	4.51
DNA	-	6.0	274	-5.7	242	-	-
[Pt(L2O)]	0.2	5.9	276	-5.4	242	0.12	0.26
	0.6	6.4	276	-5.1	241	0.45	0.68
	1.0	6.7	276	-5.2	240	0.72	0.54
	2.0	7.0	275	-4.1	240	1.00	1.64

a = (degrees · cm^2 · $dmol^{-1}$) x 10^3

Table 1. Maximums and minimums of ellipticity (θ) at different ratio [metal]/[DNA] and their corresponding wavelengths.

The ligand L2OH do not modify significantly the ellipticity of the bands neither the maximum nor minimum of wavelengths (Fig. 7a), Table 1), consequently the secondary structure of DNA is not modified.

[PdCl(L2O)] complex (Fig. 7b)) has a big effect in DNA secondary structure, when complex concentration increases the positive ellipticity also improves while the negative band decreases (Table. 1). This change suggests that this complex can modify DNA B form to A form. CD spectra show also a displacement in the wavelength to higher values, when the complex concentration increases. This is called bathochromic effect[20].

The modification of the ellipticity of DNA with increasing concentrations of [PtCl(L2O)] (Fig. 7c), Table. 1) indicates only a slightly distortion of its secondary structure.

5.3.3. Gel electrophoresis

The DNA-cleaving properties of the complexes where investigated for pBR322 plasmid DNA (15 μM in base pairs) and different concentrations of the compounds (0-100 μM) in cacodylate-NaCl buffer incubated at 37ºC for 24 hours. A loading buffer of xylene cyanol (4 μL) was added and the mixture was subjected to electrophoresis gel of agarose in TBE buffer (Tris-borate-EDTA) at 100V for 1 hour. After, the gel was placed in a container with 150 mL of TBE and 15 μL of display buffer (SYBR Safe) and was agitated overnight.

Electrophoresis in agarose gel is one of the most used methods to separate and identify DNA fragments. Pure plasmid DNA gives two bands (lanes 6, 13, 20, 27), the most intense one corresponding to supercoiled DNA (form I), while the second one is ascribed to the circular nicked form (form II). There is another form which is linear (form III) and migrates between form I and II[17].

The agarose gel electrophoresis of L2OH, [PdCl(L2O)], [PtCl(L2O)] and [$PtCl_2$(L2OH)] are shown in Fig. 8 respectively. The compounds concentrations are 5, 10, 25, 50 and 100 μM.

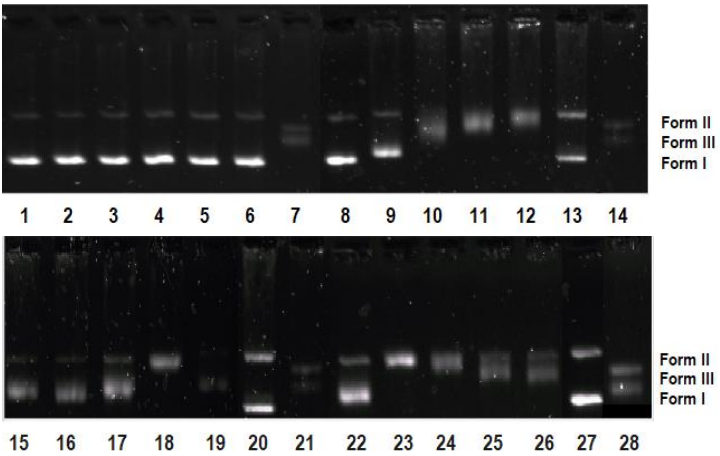


Figure 8. Agarose gel electrophoresis images of pBR322 plasmid DNA incubated for 24 h at 37ºC in cacodylate-NaCl buffer with increasing concentration of compounds. Lane 1-5 **L2OH** (5-100 μM); lane 6, 13, 20, 27 **DNA** control; lane 7, 14, 21, 28 **cisplatin** (10 μM); lane 8-12 **[PdCl(L2O)]** (5-100 μM); lane 15-19 **[PtCl(L2O)]** (5-100 μM); lane 22-26 **[PtCl2(L2OH)]** (5-100 μM).

Incubation of DNA with cisplatin affects the electrophoretic mobility of both form I and form II; cisplatin-mediated unwinding of form I causes a diminution of its mobility, whereas the unwinding of form II increases its mobility because of a shortening effect on the DNA contour length (see lanes 7, 14, 21 and 28). Free ligand L2OH (Fig. 8 Lane 1-5) does not affect the structure of the DNA molecule.

The gel electrophoresis results for [PdCl(L2O)] are shown in (Fig. 8 Lane 8-12). For [complex] > 10 μM complex seems to induce the unwinding of form I, converting it into form II. This may be explained by single-strand cleavage of the plasmid upon platinum binding.

For [PtCl(L2O)] (Fig. 8 Lane 15-19), the electrophoresis images shown that the form II is the predominant in low concentrations and for [complex] > 50 μM it is seen that DNA is almost broken.

The electrophoresis of [$PtCl_2$(L2OH)] (Fig. 8 Lane 22-26) show that in lower concentrations DNA do not change the DNA form, in a concentration of 10 μM DNA is in form II and increasing the concentrations until 100 μM DNA changes to form III.

5.3.4. UV-Vis spectroscopy

The interaction of the ligand L2OH and the complexes [MCl(L2O)] and [$PtCl_2$(L2OH)] with DNA was investigated by UV-Vis spectrophotometric titration. Experiments were carried out by addition of increasing amounts of DNA (from 0 to 100 μM, in base pairs) to a 25 μM solution of the compounds in a cacodylate-NaCl buffer. All samples were incubated for 24 hours at 37ºC. The spectra were recorded from 200 to 800 nm.

To compare the DNA-binding strength of the different compounds, their intrinsic binding constant K_b was calculated using Eq. 2.

$$\frac{[\mathrm{DNA}]}{\varepsilon_\mathrm{a}-\varepsilon_\mathrm{f}}=\frac{[\mathrm{DNA}]}{\varepsilon_0-\varepsilon_\mathrm{f}}+\frac{1}{\mathrm{K_b}(\varepsilon_0-\varepsilon_\mathrm{f})}$$

Equation 2.

Where ε_a is the extinction coefficient of the formed DNA-complex (Abs_{obs}/[complex]), ε_f is the extinction coefficient of the free complex in solution and ε_0 is the extinction coefficient for the compound in the fully DNA-bound form. The intrinsic binding constant K_b were determined from the corresponding plots of [DNA]/(ε_a - ε_f) versus [DNA].

The UV-Vis spectrum of [PdCl(L2O)] and its [DNA]/(ε_a - ε_f) versus [DNA] plot are shown in Fig. 9. The rest of the spectres are in the Appendix 9.

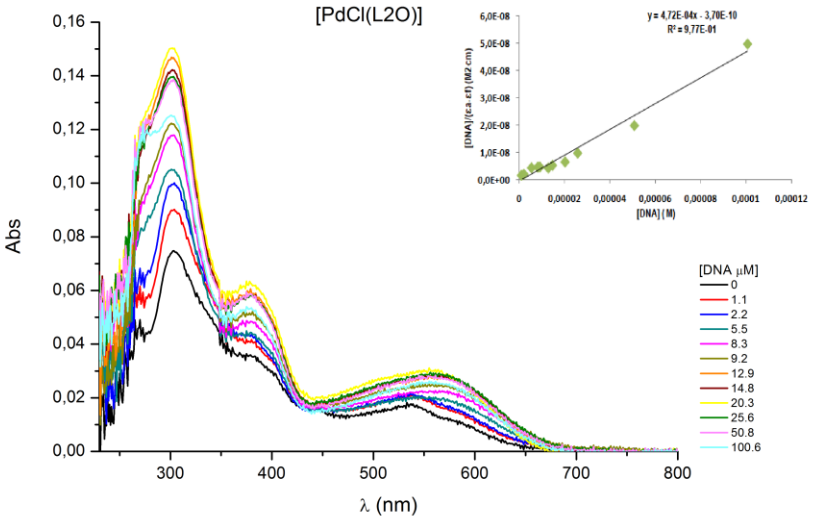


Figure 9. [PdCl(L2O)] UV-Vis spectra and [DNA]/(εa - εf) *vs* [DNA].

The binding constant (K_b) of the compounds are shown in Table 2.

Compound	L2OH	[PdCl(L2O)]	[PtCl(L2O)]	[$PtCl_2$(L2OH)]
K_b	$8.5 \cdot 10^4$	$1.3 \cdot 10^6$	$1.6 \cdot 10^5$	$3.8 \cdot 10^6$

Table 2. Compounds K_b.

The complexes that present a major interaction with DNA are [PdCl(L2O)] and [$PtCl_2$(L2OH)], also [PtCl(L2O)] interacts with DNA but with a lower K_b. The ligand interacts with DNA but as it was expected it has the lowest K_b[18].

6. EXPERIMENTAL SECTION

Elemental analysis (C, H and N) were made in Serveis Cientifico-Tècnics de la Universitat de Barcelona. NMR experiments (proton, carbon and two-dimensional {1H-13C}-HQSC-NMR) were been done with Mercury 400MHz spectrometer. Infrared spectra were obtained using FT-IR Thermo Nicolet 330, in a 4000-400 cm-1. UV-Vis experiments were performed with a Varian Cary-100 spectrophotometer. Fluorescence measurements were made with Nanolog-Horiba Jobin Yvon. Circular dichroism titrations were made with an A JASCO J-815 circular dichroism spectropolarimeter. The X-rays diffraction were collected on a Bruker APEX II QUAZAR diffractometer equipped with a microfocus multilayer monochromator with MoKα radiation (λ = 0.71073 Å). Electrophoresis images were acquired using a Gel Doc EZ Imager instrument.

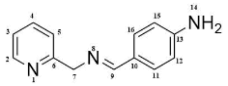
6.1. MATERIALS AND METHODS

Na_2PdCl_4 and $[PtCl_2(DMSO)_2]$ were been prepared following literature processes [21, 22]. The rest of reagents and solvents were obtained commercially (Arcos Organics and Sigma Aldrich) and were used without further purification.

6.2. PREPARATION OF LIGANDS

6.2.1. Preparation of (*E*)-4-(((pyridine-2-ylmethyl)imino)methyl)aniline (L1)

An ethanolic solution (10mL) of 125mg (1.03 mmol) of 4-aminobenzaldehyde was added to a solution of 112mg (1.03 mmol) of 2-picolylamine in ethanol (10mL). The resulting solution was refluxed for 7 hours. After this time, it was filtered and concentrated to dryness on a rotator evaporator.

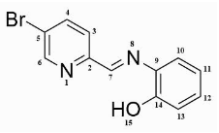


Orange solid. Yield: 183mg [84%]. Analysis calculated for $C_{13}H_{13}N_3 \cdot 0.4EtOH$(%): C 72.16, H 6.77, N 18.29; found: C 70.2, H 6.7, N 18.3. IR (KBr cm^{-1}): 3329 (ν(NH)), 1600 (ν(C=N)). ^1H NMR (DMSO, 400 MHz, ppm): δ 8.48 (dd, J= 4.0 Hz, 1H, H^2), 8.23 (s, 1H, H^9), 7.73 (td, J= 8.0 Hz, 1H, H^4), 7.43 (d, J= 8.0 Hz, 2H, H^{11}, H^{16}), 7.34 (d, *J*= 8.0 Hz, 1H, H^5), 7.22 (td, *J*= 4.0 Hz, 1H, H^3), 6.55 (d, *J*= 8.0 Hz, 2H, H^{12}, H^{15}), 5.6 (s, 2H, H^{14}), 4.71 (s, 2H, H^7). ^{13}C NMR (DMSO, 101 MHz, ppm): δ 162.9 (C^9), 160.2

(C^6), 152.0 (C^{13}), 149.3 (C^2), 137.1 (C^4), 130.1 (C^{11}, C^{16}), 124.3 (C^{10}), 122.6 (C^5), 122.4 (C^3), 113.7 (C^{12}, C^{15}), 66.3 (C^7).

6.2.2 Preparation of (*E*)-2-(((5-bromopyridin-2-yl)methylene)amino)phenol) (L2OH)

An ethanolic solution (10mL) of 125mg (0.68 mmol) of 5-bromopicolinaldehyde was added to a solution of 73mg (0.68 mmol) of 2-aminophenol. The resulting solution was refluxed for 2 hours and concentrated to dryness on a rotator evaporator.

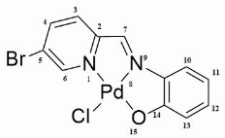


Orange solid. Yield: 342mg [92%]. Analysis calculated for $C_{12}H_9BrN_2O$(%): C 52.01, H 3.27, N 10.11; found: C 51.8, H 2.9, N 10.1. IR (KBr cm^{-1}): 3349 (v(O-H)), 1585 (v(C=N)). ^{1}H NMR (DMSO, 400 MHz, ppm): δ 9.32 (s, 1H, H^{15}), 8.86 (d, J=2.0 Hz, 1H, H^6), 8.73 (s, 1H, H^7), 8.39 (d, J= 8.0 Hz, 1H, H^4), 8.25 (dd, J= 2.0, 8.0 Hz, 1H, H^3), 7.35 (d, *J*= 4.0, 8.0 Hz, 1H, H^{13}), 7.17 (t, *J* =4.0, 8.0 Hz, 1H, H^{12}), 6.95 (d, *J*= 4.0, 8.0 Hz, 1H, H^{10}), 6.88 (t, J= 4.0, 8.0 Hz, 1H, H^{11}). ^{13}C NMR (DMSO, 101 MHz, ppm): δ 158.3 (C^7), 153. 7 (C^{14}), 152.1 (C^6), 150.6 (C^2), 140.0 (C^4), 136.7 (C^9), 129.1 (C^{12}), 123.6 (C^5), 122.5 (C^3), 120.1 (C^{13}), 120.0 (C^{11}), 116.8 (C^{10}).

6.3. PREPARATION OF COMPLEXES

6.3.1. Synthesis of [PdCl(L2O)]

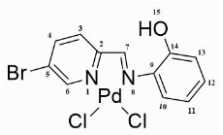
A methanolic solution (10mL) of L2OH (100mg, 0.360 mmol) was added drop by drop to a methanolic solution of Na_2PdCl_2 (106mg, 0.360 mmol) which was filtered previously. The solution was stirred for 1 hour and the stoichiometric amount of NEt_3 was added. After 1 hour of stirring the resulting precipitate was isolated.



Purple solid. Yield: 112mg [75%]. Analysis calculated for $[PdCl(C_{12}H_8BrN_2O)]\cdot 0.5CH_3OH\cdot H_2O$(%): C 33.21, H 2.65, N 6.20; found: C 33.0, H 2.4, N 6.3. IR (KBr cm^{-1}): 1576 (v(C=N)). ^{1}H NMR (DMSO, 400 MHz, ppm): δ 8.45 (dd, J= 8.0, 2.0 Hz, 1H, H^3), 8.43 (s, 1H, H^7), 8.32 (d, J= 2.0 Hz, 1H, H^6), 7.70 (d, J= 8.0 Hz, 1H, H^4), 7.38 (d, *J*= 8.0 Hz, 1H, H^{13}), 7.07 (t, *J*= 8.0 Hz, 1H, H^{12}), 6.49 (m, 2H, H^{10}, H^{11}). ^{13}C NMR (DMSO, 101 MHz, ppm): δ 175.7 (C^7), 159.6 (C^6), 151.1 (C^2), 150.7 (C^{14}), 143.7 (C^4), 135.1 (C^9), 134.2 (C^{12}), 128.1 (C^3), 121.9 (C^{10}), 119.6 (C^{11}), 118.6 (C^5), 115.7 (C^{13}).

6.3.2. Synthesis of [$PdCl_2$(L2OH)]

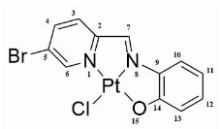
The stoichiometric amount of HCl is added to a methanolic solution (10mL) of Na_2PdCl_2 (106mg, 0.360 mmol) filtered previously. This solution is added drop by drop to a methanolic solution (10mL) of L2OH (100mg, 0.360 mmol). The resulting solution is stirred for 1 hour and filtered.



Orange solid. Yield: 78mg [48%]. Analysis calculated for [$PdCl_2(C_{12}H_9BrN_2O)$](%): C 31.72, H 2.0, N 6.16; found: C 31.3, H 2.2, N 5.9. IR (KBr cm^{-1}): 3274 (v(O-H)), 1593 (v(C=N)). ^{1}H NMR (DMSO, 400 MHz, ppm): δ 10.12 (s, 1H, H^{15}), 9.03 (d, J= 4.0 Hz, 1H, H^6), 8.69 (dd, J= 4.0, 8.0 Hz, 1H, H^3), 8.69 (s, 1H, H^7), 8.12 (d, J= 8.0 Hz, 1H, H^4), 7.17 (m, 2H, H^{10}, H^{12}), 6.90 (d, *J*= 8.0 Hz, 1H, H^{13}), 6.82 (t, J= 8.0 Hz, 1H, H^{11}).

6.3.3. Synthesis of [PtCl(L2O)]

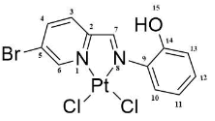
A methanolic solution (10mL) of L2OH (70mg, 0.253 mmol) was added to a solution of *cis*-[$PtCl_2(DMSO)_2$] (107mg, 0.253 mmol, in 20mL of methanol), which was previously refluxed and filtered. The resulting solution was stirred for 30 minutes and the stoichiometric amount of NEt_3 was added. After 1 hour the resulting precipitate was isolate.



Green solid. Yield: 57mg [45%]. Analysis calculated for [$PtCl(C_{12}H_8BrN_2O)]\cdot 0.5H_2O$(%): C 27.95, H 1.79, N 5.43; found: C 27.8, H 1.6, N 5.3. IR (KBr cm^{-1}): 1591 (v(C=N)). ^{1}H NMR (DMSO, 400 MHz, ppm): δ 8.83 (s, $^3J_{H\text{-}Pt}$= 100.0 Hz, 1H, H^7), 8.53 (d, 1H, H^6), 8.44 (dd, J= 8.0 Hz, 1H, H^3), 7.62 (d, J= 8.0 Hz, 1H, H^4), 7.40 (d, *J*= 8.0 Hz, 1H, H^{10}), 7.05 (t, *J*= 8.0 Hz, 1H, H^{12}), 6.62 (d, J= 12 Hz, 1H, H^{13}), 6.52 (t, J= 12.0 Hz, H^{11}). ^{13}C NMR (DMSO, 101 MHz, ppm): δ 178.1 (C^2), 161.4 (C^{14}), 153.1 (C^7), 150.3 (C^6), 143.5 (C^3), 135.4 (C^9), 135.0 (C^{12}), 128.8 (C^4), 123.3 (C^5), 119.2 (C^{10}), 118.6 (C^{13}), 115.7 (C^{11}).

6.3.4. Synthesis of [$PtCl_2$(L2OH)]

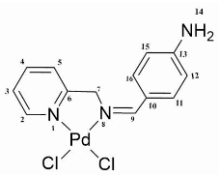
The stoichiometric amount of HCl was added to a methanolic solution (20mL) of *cis*-[$PtCl_2(DMSO)_2$] (30mg, 0.307 mmol) which was refluxed and filtered previously. This solution was added drop by drop to a solution of L2OH (85mg, 0.307 mmol) in 10mL of methanol, then the solution was stirred for 2 hours. The resulting solution is allowed to crystallize at room temperature.

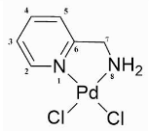


Crystalline black solid. Yield: 45mg [27%]. Analysis calculated for $[PtCl_2(C_{12}H_9BrN_2O)]\cdot 0.5H_2O$(%): C 26.54, H 1.67, N 5.16; found: C 26.6, H 1.8, N 5.0. IR (KBr cm^{-1}): 3311 (ν(O-H)), 1651 (ν(C=N)). ^{1}H NMR (DMSO, 400 MHz, ppm): δ 10.15 (s, 1H, H^{15}), 9.51 (d, J= 1.6 Hz, 1H, H^6), 9.30 (s, 1H, H^7), 8.76 (dd, J= 1.6, 8.0 Hz, 1H, H^3), 8.17 (d, J= 8.0 Hz, 1H, H^4), 7.21 (m, 2H, H^{10}, H^{12}), 6.97 (d, *J*= 12.0 Hz, 1H, H^{13}), 6.88 (t, J= 8.0 Hz, 1H, H^{11}). ^{13}C NMR (DMSO, 101 MHz, ppm): δ 173.4 (C^7), 155.9 (C^2), 151.2 (C^{14}), 149.8 (C^6), 143.2 (C^3), 141.5 (C^9), 130.4 (C^4), 130.1 (C^{12}), 126.1 (C^{10}), 120.1 (C^5), 118.1 (C^{11}), 116.3 (C^{13}).

6.3.5. Synthesis of $[PdCl_2(L1)]$

A methanolic solution (10mL) of L1 (60mg, 0.284 mmol) was added drop by drop to a solution of Na_2PdCl_2 (84mg, 0.248 mmol) which was filtered previously. The solution was stirred for 30 minutes and the resulting precipitate was isolated.





Orange solid.

$[PdCl_2(L1)]$ ^{1}H NMR (DMSO, 400 MHz, ppm): δ 8.90 (d, J= 8.0 Hz, 1H, H^2), 8.40 (s, 1H, H^9), 8.10 (t, 1H, H^4), 7.71 (d, J= 8.0 Hz, 2H, H^{11}, H^{16}), 7.53 (m, 2H, H^3, H^5), 6.69 (m, J= 8.0 Hz, 2H, H^{12}, H^{15}), 5.60 (s, 2H, H^{14}), 5.54 (s, 1H, H^7).

[Pd(2-plycolylamine)] ^{1}H NMR (DMSO, 400 MHz, ppm): δ 8.76 (d, J= 8.0 Hz, 1H, H^2), 8.05 (t, J= 8 Hz, 1H, H^4), 7.62 (d, J= 8.0 Hz, 1H, H^3), 7.51 (t, J= 8.0 Hz, H^5), 5.62 (s, 1H, H^8).

7. CONCLUSIONS

Two new Schiff bases [(*E*)-4-(((pyridine-2-ylmethyl)imino)methyl)aniline (**L1**) and (*E*)-2-(((5-bromopyridin-2-yl)methylene)amino)phenol (**L2OH**)] were synthesized and characterized. The reaction of L2OH with Na_2PdCl_4 and $[PtCl_2(DMSO)_2]$ allows to obtain pure palladium (II) and platinum (II) complexes [MCl(L2O)] and $[MCl_2(L2OH)]$ optimizing the reaction's pH. The characterization data of these compounds agree with the proposed structures.

Fluorescence studies indicate that any of the complexes prepared cannot displace ethidium bromide (EB) so the Stern–Volmer quenching constant (K_{sv}) between EB and DNA is bigger than the synthesised complexes and DNA.

Circular dichroism spectra show that the ligand L2OH does not change the secondary structure of DNA whereas the [MCl(L2O)] complexes do it. The palladium complex, [PdCl(L2O)], causes the biggest modification.

All the metallic complexes studied modify the mobility of the DNA as can be seen in the electrophoresis studies and some of they can even break it at high concentrations.

UV-Vis studies show that the complexes that have more interaction with DNA are [PdCl(L2O)] and $[PtCl_2(L2OH)]$ and as it was expected the ligand has less interaction than the complexes.

In general, all the studies carried out indicate that the complexes which have major interaction with DNA are [PdCl(L2O)] and $[PtCl_2(L2OH)]$ even thought more studies must be done.

8. REFERENCES AND NOTES

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9. ACRONYMS

CD – Circular dichroism

d – doublet

dd – doublet of doublets

DMSO – Dimethyl sulfoxide

DNA – Deoxyribonucleic Acid

HSQC – heteronuclear single quantum correlation

IR – Infrared Spectroscopy

m – multiplet

NMR – Nuclear Magnetic Resonance

Pd – palladium

Pt – platimun

s – singlet

t – triplet

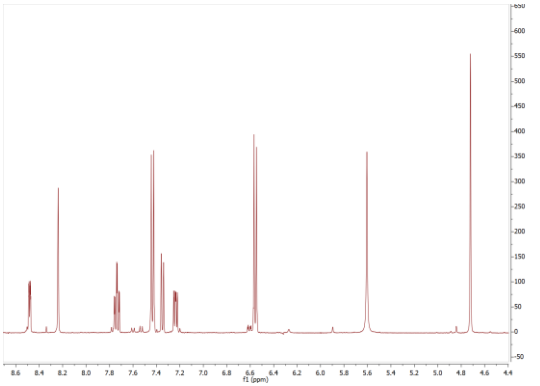
TBE – Tris-borate-EDTA

UV-Vis – Ultraviolet-visible

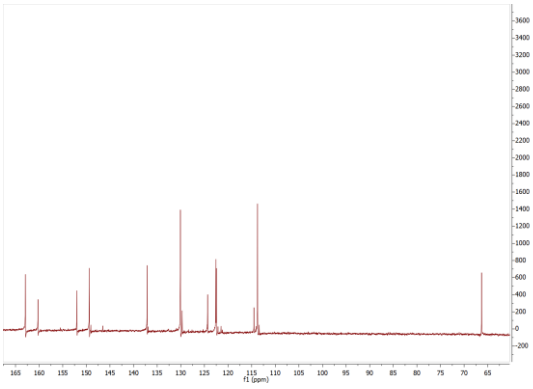
APPENDICES

APPENDIX 1: SPECTRA OF L1

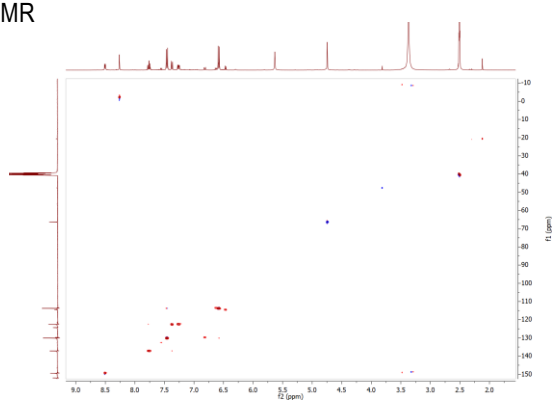
^{1}H NMR (DMSO)



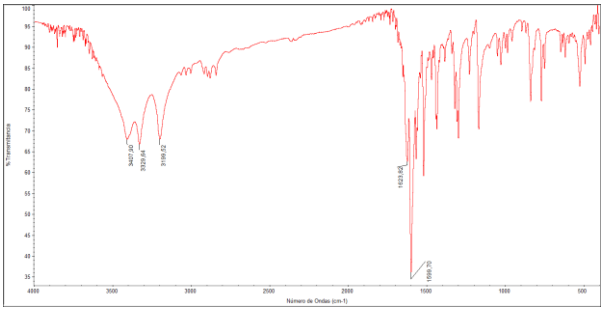
^{13}C NMR (DMSO)



HSQC-NMR

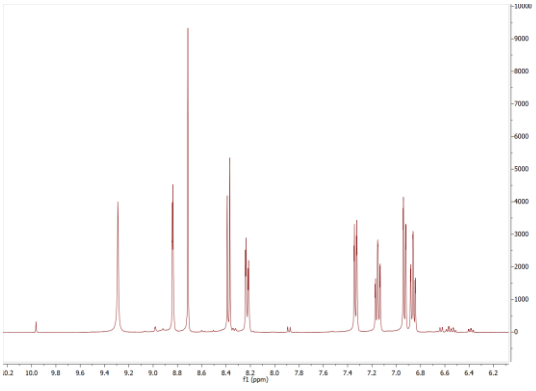


IR (KBr)

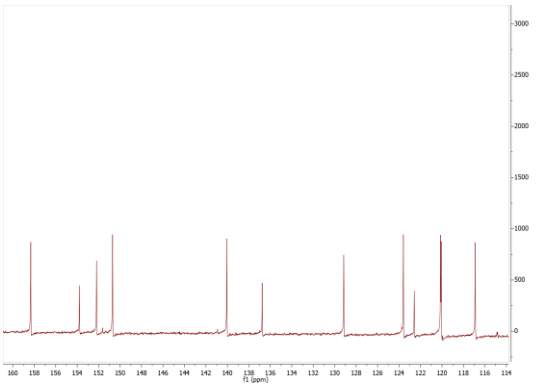


APPENDIX 2: SPECTRA OF L2OH

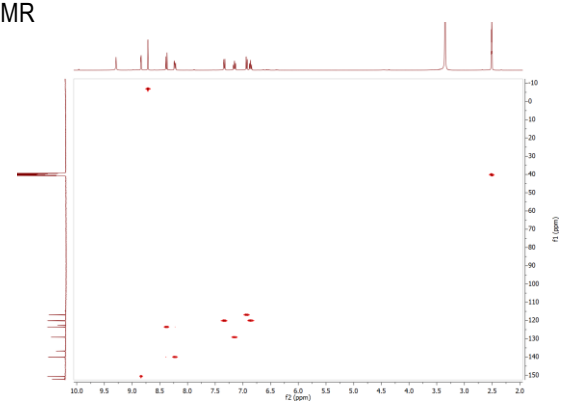
^{1}H NMR (DMSO)



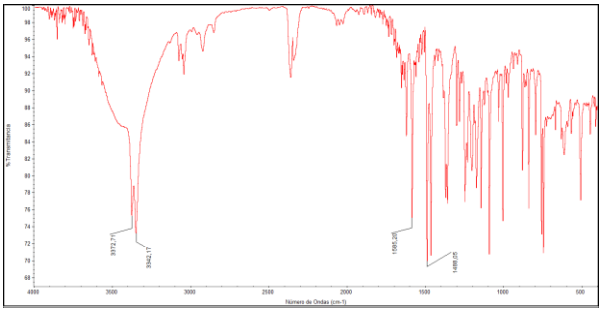
^{13}C NMR (DMSO)



HSQC-NMR



IR (KBr)

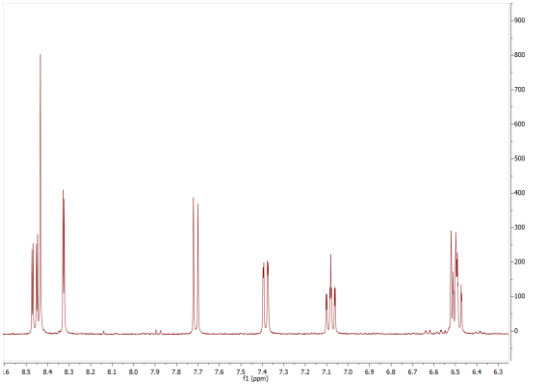


APPENDIX 3: CRYSTALLOGRAPHIC PARAMETERS OF L2OH

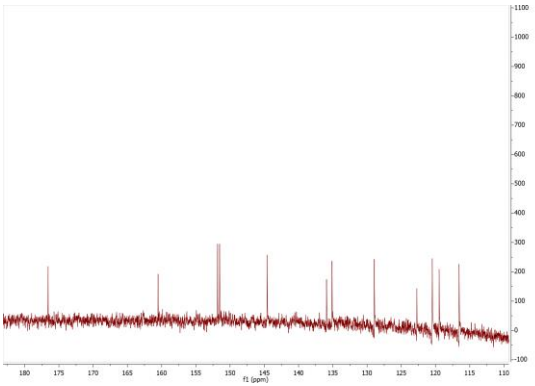
Compound	L2OH
Empirical formula	$C_{12}H_9BrN_2O$
Molecular weight (g·mol^{-1})	277.11
Temperature (K)	100(2)
Crystalline system	monoclinic
Cristal size (mm^3)	0.30x0.23x0.20
Space group	P 21/n
a (Å)	12.1970
b (Å)	4.6219
c (Å)	19.0106
α (º)	90
β (Å)	98.993
γ (Å)	90
Volume (Å^3)	1058.52(14)
Z	4
Pcalc (Mg/m^3)	1.739
μ (mm^{-1})	3.86
F (000)	552.0
Θ for data collection (º)	3.43-27.40
Reflections collected / unique	2318
Complements to theta	25.242
Data / restrints / parameters	2318/0/149
Goodness-of-fit on F2	1.079
Final R indices [I>2σ(I)]	R1 = 0.0237, wR2 = 0.0571
R indices (all data)	R1 = 0.0281, wR2 = 0.0584

APPENDIX 4: SPECTRA OF [PDCL(L2O)]

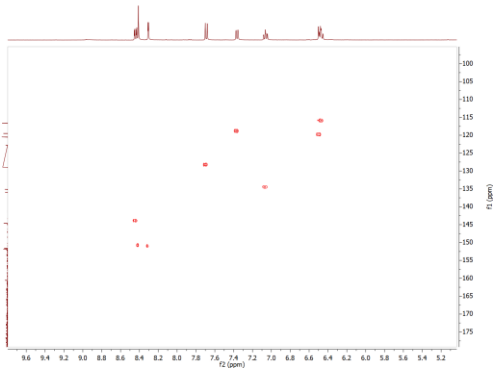
^{1}H NMR (DMSO)



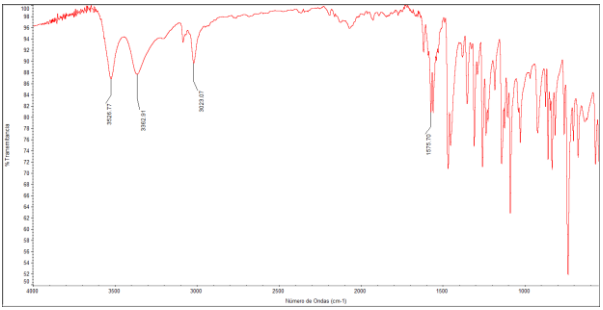
^{13}C NMR (DMSO)



HSQC-NMR

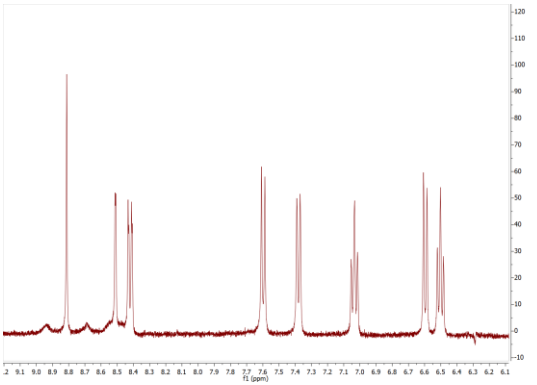


IR (KBr)

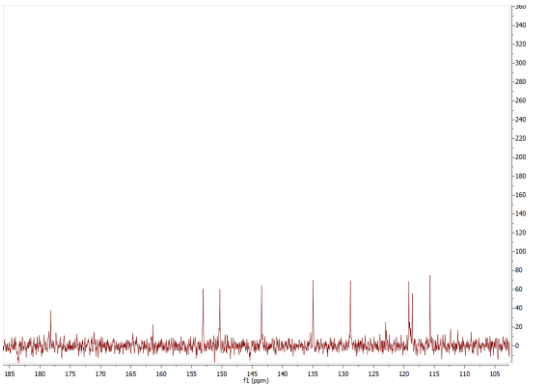


APPENDIX 5: SPECTRA OF [PTCL(L2O)]

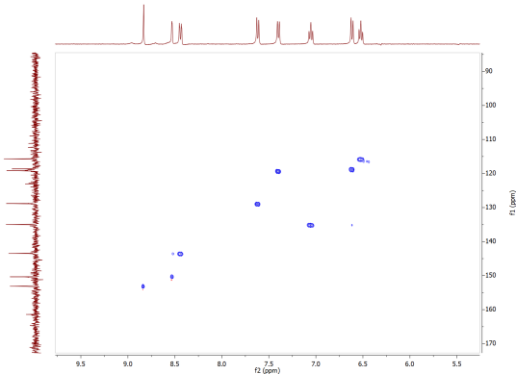
^{1}H NMR (DMSO)



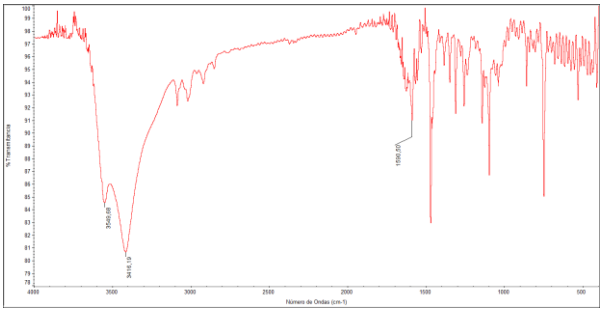
^{13}C NMR (DMSO)



HSQC-NMR

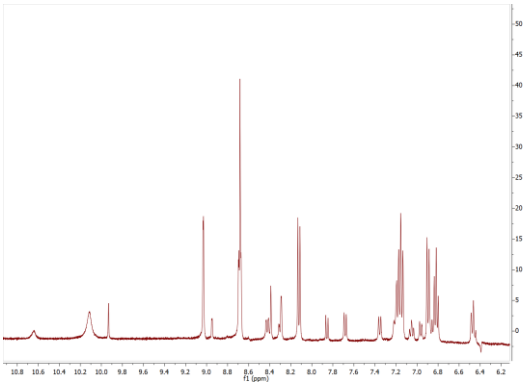


IR (KBr)

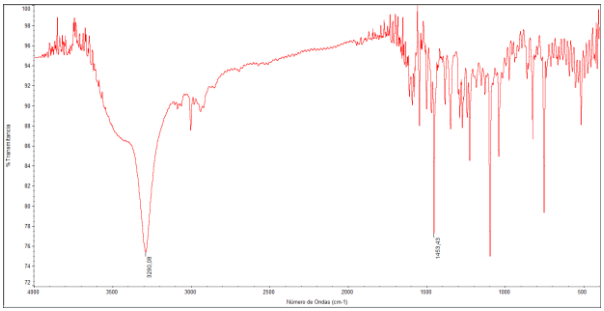


APPENDIX 6: SPECTRA OF [PDCL2(L2OH)]

^{1}H NMR (DMSO)

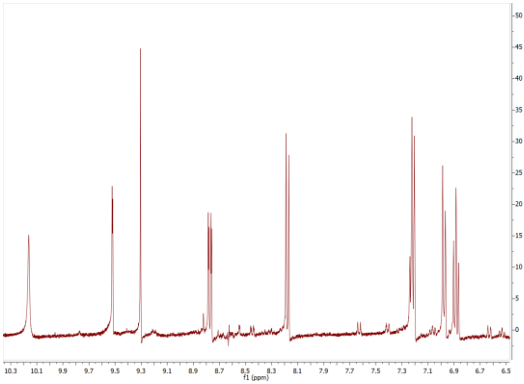


IR (KBr)

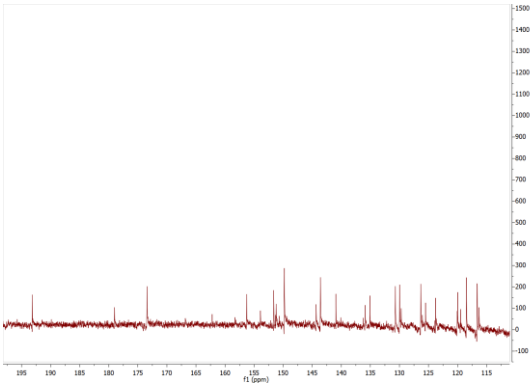


APPENDIX 7: SPECTRA OF [PTCL$_2$(L2OH)]

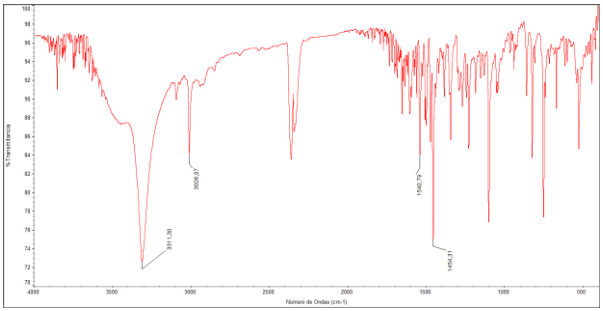
^{1}H NMR (DMSO)



^{13}C NMR (DMSO)

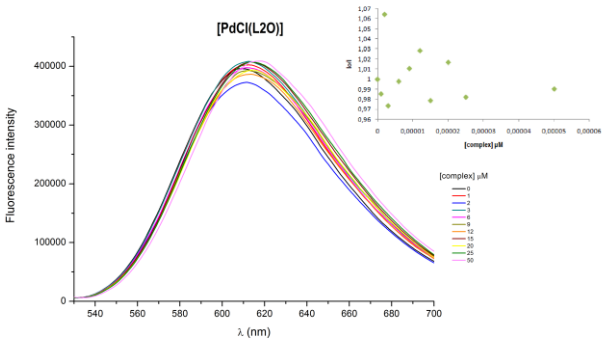


IR (KBr)

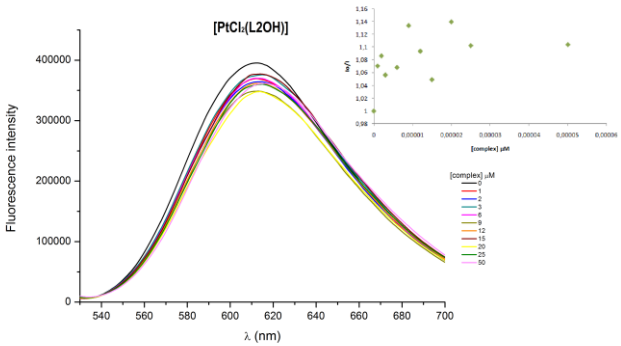


APPENDIX 8: FLUORESCENCE SPECTRA

[PdCl(L2O)]

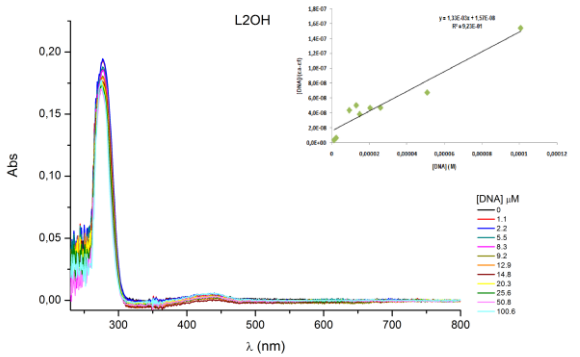


[$PtCl_2$(L2OH)]

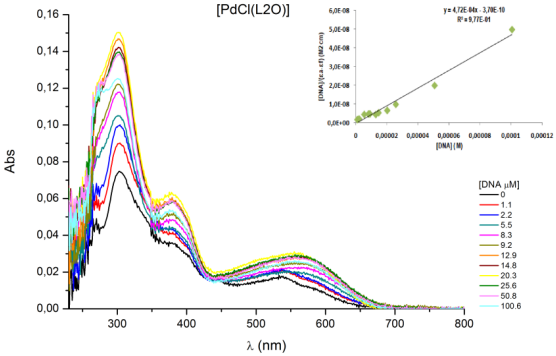


APPENDIX 9: UV-VIS SPECTRA

L2OH



[PdCl(L2O)]



[$PtCl_2$(L2OH)]